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Determination of furfurals in Manuka honey using piston-cylinder liquid–liquid extraction and gas chromatography

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ABSTRACT

A rapid analytical approach for the direct measurement of furfurals such as *2-furfural* and *5-methyl-2-furfural* at parts-per-billion level in Manuka honey is described. The approach employs a piston-cylinder based liquid–liquid extraction device using chloroform extraction solvent. This device substantially reduces extraction time by a factor of 120 times compared to solid phase micro-extraction and reduces solvent consumption by a factor of 25 times compared to liquid–liquid extraction with mechanical agitation. A recently commercialised capillary column offering a high degree of inertness permits separation and detection of the analytes at ultra-trace level without derivatisation. A three-port planar microfluidic device with a mid-point pressure is also incorporated to back-flush heavier compounds in the matrix to improve column longevity and overall system cleanliness. With this approach, analysis is conducted in less than 7 min. Repeatability of retention times for all compounds is less than 0.1% ($n = 20$). The compounds cited can be analysed over a range from 1 ng/g to 10 μg/g in honey with a 5 ng/g limit of quantification (LOQ) and correlation coefficients of at least 0.999. Relative precision is less than 2.8% RSD ($n = 20$) at 50 ng/g level with analyte extraction efficiency of greater than 99% ($n = 3$) over a range from 5 ng/g to 10 μg/g in the matrix described. The analytical system requires only minimal maintenance and is suitable for remote site deployment. Under the analytical conditions established and with a practical LOQ of 5 ng/g, 100 samples can be analysed before septum/liner/o-ring replacements are needed. As a preventive measure, the pre-column can be replaced once every six months to maintain chromatographic fidelity.

1. Introduction

Manuka honey is produced by European honeybees (*Apis mellifera* Linnaeus) feeding primarily on Manuka (*Leptospermum scoparium* J.R. Forst. & G. Forst.). Manuka honey is increasingly being employed in medical sciences to treat wounds due to its antiseptic property [1–3]. Most recently, it has been applied to treat antibiotic resistant chronic rhino sinusitis and as a nasal cavity rinse to prevent post-surgery infections [4,5]. For these medical applications, the highest quality Manuka honey, free of undesired by-products from prolonged storage or harsh shipment mishandling conditions, is preferred.

The presence of furfurals is a key indicator of the degree of freshness and quality of carbohydrate containing products such as honey. The formation of 2-furural and 5-methyl-2-furfural has been attributed to Maillard reaction in carbohydrate containing foods [6]; 2-furfural is a Maillard reaction product derived from pentose sugars while 5-methyl-2-furfural is derived from hexose sugars [7]. Determination of 2-furfural and 5-methyl-2-furfural in various matrices is commonly performed by use of high performance liquid chromatography (HPLC) with either *acetonitrile*:*water* or *methanol*:*water* mobile phase and a variable wavelength or diode array detector [8–11]. These furanic compounds have also been studied by gas chromatography (GC) [7], HPLC–mass spectrometry (with atmospheric pressure chemical ionisation), HPLC-pulsed amperometric detection, and thin-layer chromatography [12–14]. Throughout the methodologies cited, solid phase microextraction (SPME) or solid phase extraction (SPE) or liquid–liquid extraction (LLE) have been commonly employed as sample clean-up and

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pre-concentration techniques. Lengthy extraction time is a common drawback associated with the techniques previously employed for extraction of 2-furfural and 5-methyl-2-furfural from various matrices [1,6,7,9–14]. For instance, Gaspar and Lopes reported an extraction time of a minimum of 2 h using SPME [7].

In the present article, we introduce a fast and accurate ultra-trace GC approach using flame ionisation detection (FID) capable of detecting parts-per-billion of the analytes of interest in Manuka honey. Separation of analytes was conducted with a newly-introduced highly-inert capillary column thereby eliminating the need for derivatisation. A three-port planar microfluidic device with a mid-point pressure source is employed to permit back-flushing, to improve sample throughput and analytical system cleanliness.

The present approach also employs an extraction device based on the principle of piston-cylinder LLE with chloroform as an extraction solvent for matrix removal and analyte enrichment. Cais et al. invented this LLE device known as Mixxor [15]. Mixxor has been utilised in a number of unique applications including direct extraction of aromas, blood fractionation, and immunoassays [15–19]. The Mixxor LLE device has two portions: a glass reservoir and a glass vertical mixer-separator. The mixer-separator has a channel traversing the entire length of the mixer-separator in the vertical axis. The purpose of the channel is to maximise mass transfer between the two counter-current flow fluids, with the denser chemical such as chloroform in the outside and the less dense liquid like water/honey solution in the centre of the channel [15,16]. The two virtually immiscible liquids: (a) honey/water solution and (b) chloroform are added to the reservoir. The phases are thoroughly mixed, by moving the mixer-separator in and out of the reservoir, commonly referred to as “stroking” the mixer-separator. Once the extraction is completed, the upper phase is removed and the extraction phase is transferred to a sampling vial for analysis. The present article is the first report of Mixxor extraction to extract furfurals from honey.

2. Experimental

An Agilent 6890N gas chromatograph (Agilent Technologies, Wilmington, DE, USA), equipped with an Agilent G-4512A autosampler, two split/splitless inlets, and FID was employed throughout. The instrument was also equipped with a three-port SilFlow planar microfluidic device, PN#123725 (SGE Analytical Science, Australia) for back-flushing purpose. Mid-point pressure was delivered by connecting a 0.5 m × 0.25 mm-id uncoated, but deactivated fused silica capillary tubing from the second split/splitless inlet to the planar microfluidic device. Gas chromatographic conditions used are as follows: a 2 m × 0.32 mm-id × 1.8 μm DB-624UI (Agilent Technologies, Folsom, USA) was used in the first section and a 10 m × 0.32 mm-id × 1.8 μm DB-624UI (Agilent Technologies, Folsom, USA) was used in the second section. These two columns were cut from a single 60 m × 0.32 mm-id × 1.8 μm DB-624UI column which has a stationary phase comprising of 6% cyanopropyl phenyl and 94% dimethylpolysiloxane.

The column flow in the column ensemble was 6.6 mL/min helium, in constant flow mode. The inlet pressure was 12.6 psig @ 40 °C, while the auxiliary pressure was 10.0 psig @ 40 °C to deliver the flows required. During the back-flush state, the inlet pressure was lowered to 2 psig final pressure at a rate of 99 psig/min while the mid-point pressure was raised to 17 psig at a rate of 99 psig/min. The inlet temperature was 250 °C, operating in split mode at a ratio of 3:1 and equipped with an Ultra-inert liner PN# 5190-2294 (Agilent Technologies, Folsom, USA) and the injection size was 1 μL.

The temperature was programmed from 40 °C (1 min) to 260 °C @ 25 °C/min, and maintained at 260 °C for 1 min. The FID temperature was at 300 °C with hydrogen flow rate at 30 mL/min, air flow rate at 350 mL/min and nitrogen flow rate at 30 mL/min. Chromatographic data obtained were collected using ChemStation version B.03.02 (Agilent Technologies, Waldbronn, Germany). Carrier and fuel gases such as helium, air, and nitrogen used for system performance studies were acquired from Air Liquide (Edmonton, Canada). 2-Furfural (CAS 98-01-1), 5-methyl-2-furfural (620-02-0), furfuryl alcohol (98-00-0), 2-furyl methyl ketone (1192-62-7), and 5-hydroxymethyl furaldehyde (67-47-0) standards were obtained from Sigma-Aldrich (Oakville, Canada). Solvents like cyclohexane, methanol, methylene chloride, toluene, iso-propanol and chloroform, all of ACS grade, were obtained from Fisher Scientific (Edmonton, Canada). Six Manuka honey samples were provided by HoneyDoc Products Inc. (West Vancouver, Canada) for method development and performance evaluation. These samples were from different production batches, and included a sample that was rejected by quality control department due to off-taste, and a sample that had been stored in the warehouse where the air conditioning system failed and the sample was exposed to 40 °C for 4 weeks The fresh honey samples were produced by the local beekeepers (Victoria, Canada) for HoneyDoc Products Inc.; stored at 4 °C from the time immediately following their extraction from the honeycomb to the time of their analysis in less than 14 days. Prior to analysis, each sample was homogenised with an IKA/Cole-Palmer T-18 Ultra Turrax Digital Homogenizer (Cole-Palmer, Montreal, Canada) for 30 min.

A Mixxor extraction device, NBS PN# MXLK00002 (Haifa, Israel) with a nominal volume of 2 mL was used for LLE. Deionised water (5 mL) was added to 5.0 g of sample in a 20 mL I-Chem vial and the mixture sonicated without heat for 2 min with a Bransonic 3510 sonicator (Danburry, USA). A 2.0 mL aliquot of the resulting solution was transferred to the extraction device and 400 μL of chloroform was subsequently added to the extraction device. The sample was extracted for 1 min with 10 strokes of the extraction device, and the bottom layer (chloroform) was transferred to a 2 mL auto-sampler vial for analysis.

For LLE with mechanical agitation experiments, an in-house quality assurance/control analytical procedure was employed. Deionised water (50 mL) was added to 50 g of sample in a Wheaton 150 mL serum vial. 10 mL of chloroform was added and the vial was shaken with a Burrell wrist action mechanical shaker model 75 (Pittsburgh, PA, USA) for a predetermined amount of time; as described in the results and discussions section. Once the extraction emulsion settled, a 1.5 mL aliquot of the chloroform layer was removed and transferred to a 2 mL auto-sampler vial for analysis. Sample preparation procedure for sonication extraction experiments is identical to that of LLE with mechanical agitation with the only exception of using a Brandsonic 3510 sonicator in lieu of a wrist action mechanical shaker. A mixed standard of 1000 μg/mL each of 2-furfural and 5-methyl-2-furfural in chloroform was prepared for instrument/column testing and calibration. Serial dilution was made with chloroform to produce standards over the linearity range of interest. A mixed standard of 10 μg/mL 2-furfuryl alcohol, 5-methyl-2-furfural, 2-furyl methyl ketone, and 5-hydroxymethyl furaldehyde in methanol were prepared for analyte flow path inertness study.

For extraction efficiency study using different extraction techniques and other studies including repeatability and recovery, a stock solution of 1000 μg/g 2-furfural and 5-methyl-2-furfural in honey was prepared. This was accomplished by adding 0.1 g of 2-furfural and 5-methyl-2-furfural to 99.8 g of freshly harvested honey that had been screened to contain no detectable amount of furfurals. The sample was then homogenised with a IKA/Cole-Palmer T-18 Ultra Turrax Digital Homogenizer

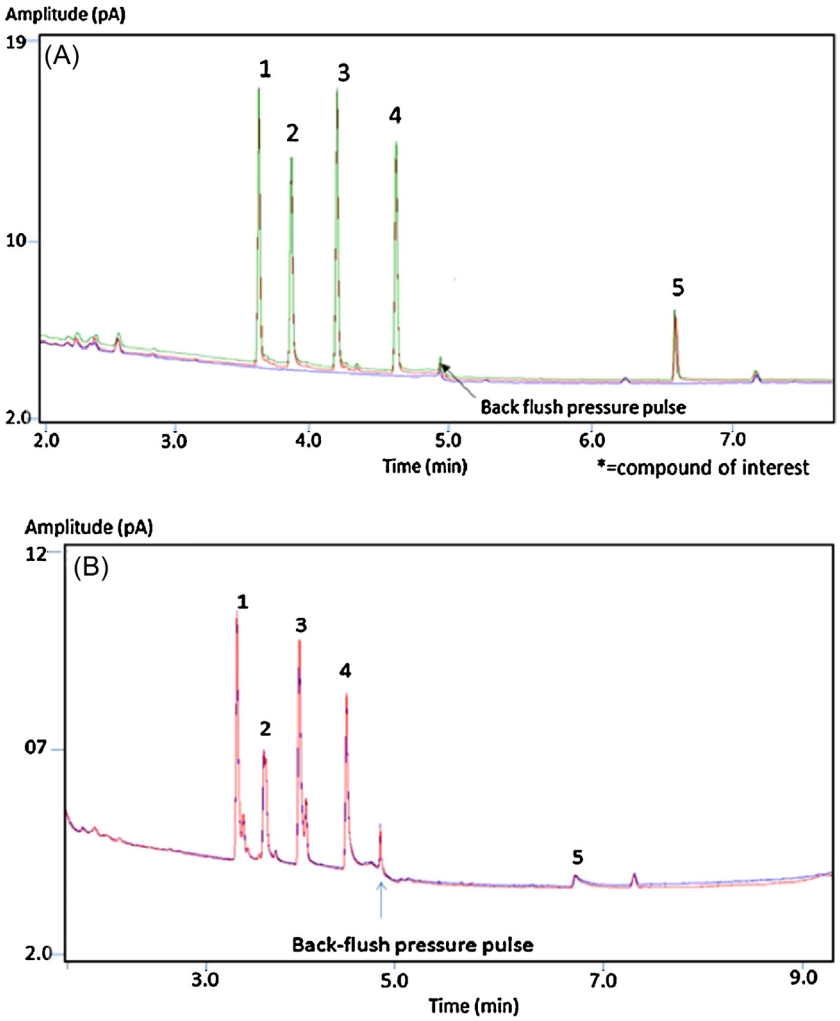

Fig. 1. (A) Overlaid chromatograms of duplicate analysis of 10 μg/mL each of a mixture of furfurals standard in methanol and a solvent blank. (B) The same sample analysed using a 15 m × 0.32 mm-id × 2 μm DB-1 column.

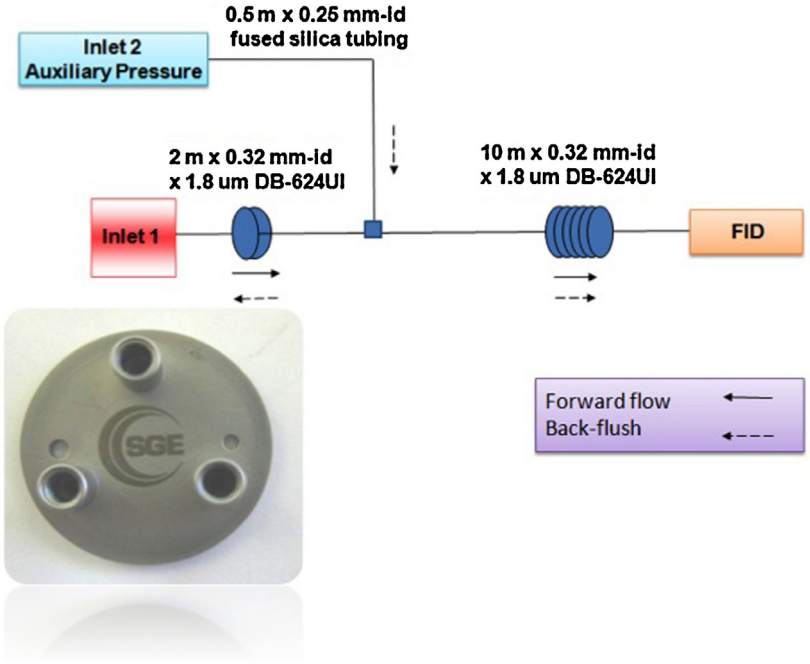

Fig. 2. Schematic diagram of the analytical system.

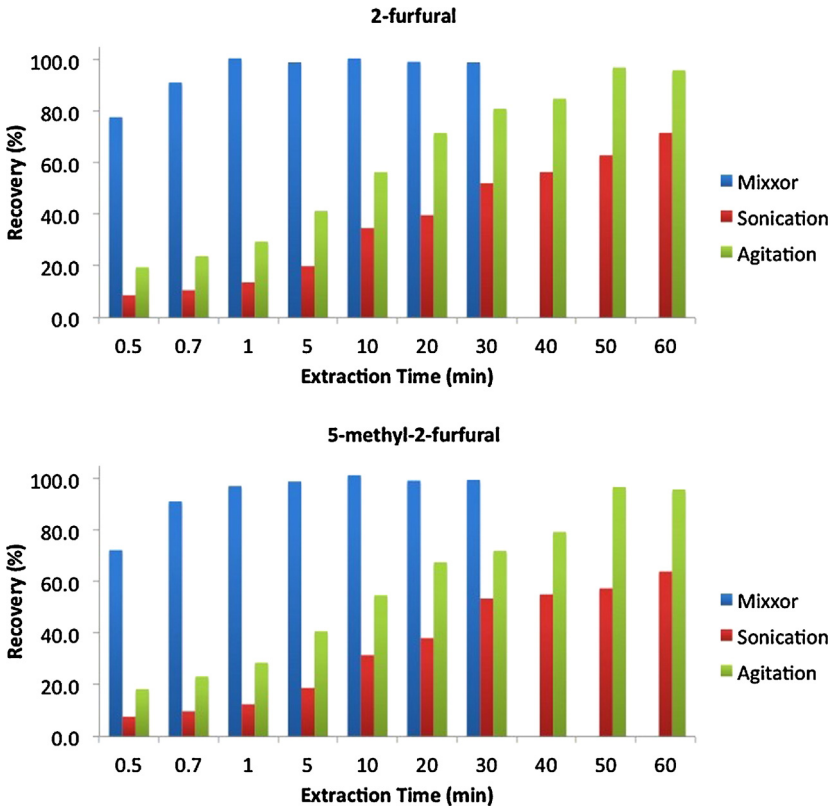

Fig. 3. (A) Extraction efficiency of 2-furfural with different extraction techniques. (B) Extraction efficiency of 5-methyl-2-furfural with different extraction techniques.

(Cole-Palmer, Montreal, Canada) for 10 min. Serial dilution by weight was made with the same honey to obtain standards over a concentration range from 5 ng/g to 20 μg/g.

3. Results and discussions

Underivatised furfurals can be problematic to analyse by GC at parts-per-billion level. Free silanol groups, impurities, and active sites on either the inner surface of the fused silica column or in the stationary phase can lead to loss of peak symmetry. Thus a DB-624UI capillary column was chosen for this application since it is demonstrably highly inert, and suited for analysis of active compounds [20]. Fig. 1A shows an overlay of chromatograms of duplicate analyses of a mixture of five furfurals (each 10 μg/mL in methanol). Chromatographic performance is excellent with peak symmetries greater than 0.92 for the both 2-furfural and 5-methyl-2-furfural. In contrast, Fig. 1B shows an overlay of chromatograms of the same mixture on a 15 m × 0.32 mm-id × 2 μm DB-1 column. Here the chromatographic results are undesirable with severe peak tailing apparent for all test compounds. Results for more polar

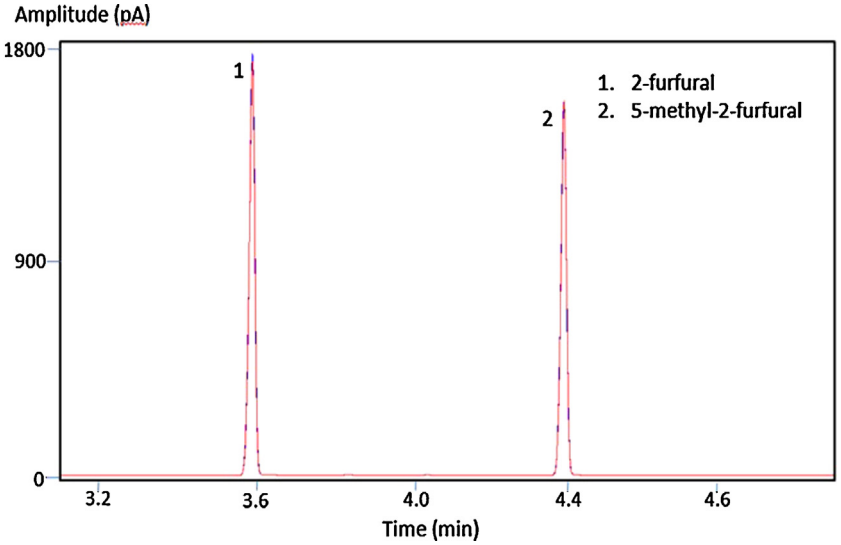

Fig. 4. Overlaid chromatograms of two extractions of 200 μg/g 2-furfural and 5-methyl-2-furfural standard mixture compared to 1000 μg/g 2-furfural and 5-methyl-2-furfural standard mixture in honey matrix.

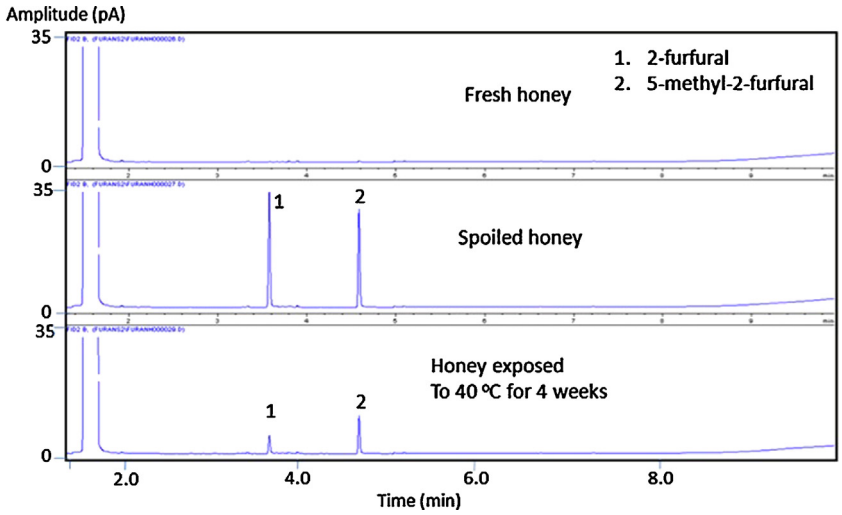

Fig. 5. Overlaid chromatograms of fresh, spoiled, and honey exposed to 40 °C for 4 weeks.

furfuryl alcohol and 5-hydroxymethyl furaldehyde, are especially poor. While the results shown in Fig. 1A are satisfying, maintaining system cleanliness is critically important to avoid deterioration of chromatographic performance. Thus a simple back-flush approach was developed to protect the analytical column from introduction of undesired components [21]. This approach involves the use of a short 2 m primary column coupled to a 10 m secondary column with a three-port planar microfluidic device. A mid-point pressure was introduced at the three-port planar microfluidic device to achieve the back-flushing result. The instrument configuration is illustrated in Fig. 2. The 2 m primary column acts as a guard column with the main purpose to retain heavier components. Once the analytes of interest reach the analytical column, the mid-point pressure is increased to 17 psig. Simultaneously, the inlet pressure is decreased to 2 psig and the residual material is back-flushed to vent while separation of the analytes proceeds on the analytical column. Back-flushing has three substantial benefits: (i) back-flushing protects the analytical column from introduction of undesired components, (ii) back-flushing reduces exposure of the stationary phase to excessive temperature programming otherwise needed to remove strongly retained matrix components, and (iii) back-flushing improves sample throughput. With this back-flushing-MDGC design, a complete analysis of 2-furfural and 5-methyl-2-furfural can be conducted in less than 7 min.

The Mixxor extraction device provides high extraction efficiency with minimal optimisation. Extraction variables in this investigation include the ratio of volume of sample to the volume of extraction solvent, type of extraction solvent, stroke rate (number of strokes/min), and extraction time (min). A 5:1 volume ratio of sample to extraction solvent was employed for all experiments in this investigation. 10 piston strokes/min with an extraction time of 1 min was found to be optimal for the application described. The maximum nominal volume (2 mL) of sample was utilised and 0.4 mL of extraction solvent was chosen primarily for practical reasons. While a smaller volume theoretically leads to a higher concentration factor, use of smaller volumes introduces solvent handling difficulties. Further, when a smaller volume of extraction solvent is used, the solubility of the solvent in the water/honey solution can have a negative impact on the overall recovery of the analyte.

Solvent selection is an important optimisation criterion. A simple investigation showed that use of chloroform provided better extraction efficiency of the target analytes than hexane, cyclohexane, methylene chloride, and toluene. All further experiments were carried out using chloroform, and exhaustive extraction was obtained in 1 min (10 piston strokes) for 2-furfural and 5-methyl-2-furfural over a range of concentrations from 5 ng/g to 10 μg/g. This is 120 times faster (1 min versus 120 min) than extracting the same analytes with the direct immersion SPME method [7].

The extraction efficiency of 2-furfural and 5-methyl-2-furfural in honey solution with the Mixxor LLE device was compared to wrist-action mechanical agitation and sonication. It took more than 60 min for mechanical agitation to achieve the same extraction efficiency of the LLE device. The Mixxor LLE approach consumed 25 times less solvent than liquid–liquid extraction with wrist-action mechanical agitation. Since a small amount of solvent was employed, this minimises solvent cost and more significantly, the cost of used solvent disposal which has increased substantially over the last decade. Sonication extraction for 60 min only achieved extraction efficiency of about 68%, with a substantial amount of emulsion formation that took more than 10 min to settle. Fig. 3A plots the results obtained for three extraction techniques using a 1000 μg/g 2-furfural in honey. Similar profiles were also obtained for 5-methyl-2-furfural as shown in Fig. 3B. Extraction efficiency approaching 100% was attained for 2-furfural and 5-methyl-2-furfural using the Mixxor LLE approach. Overlaid chromatograms of two extractions of 200 μg/g 2-furfural and 5-methyl-2-furfural standard mixture in honey compared to that of 1000 μg/g 2-furfural and 5-methyl-2-furfural standard mixture in the same matrix are presented in Fig. 4. The 5:1 concentration factor for both furfurals is clearly apparent, with no distinguishable difference in peak area from the three injections.

Analytical figures of merit for the LLE-back-flushing GC approach are highly satisfactory. Repeatability of retention times for all compounds were found to be less than 0.1% ($n = 20$) over a period of two days. Both target analytes can be analysed over a range from 1 ng/g to 10 μg/g in honey with a practical LOQ of 5 ng/g and correlation coefficients of at least 0.999. The "practical" LOQ was set as a quantitative limit that can be easily attained using different analytical system hardware and by analysts with different levels of experience in chromatography. Apart from the regular replacement of liners and septa at a rate of 100 samples per replacement, no major analytical system maintenance such as the replacement of the guard column is required for at least six months. It is reasonable to expect further improvement in LOQ by combining the current approach with selective detection such as mass spectrometry in selected ion monitoring mode.

Fig. 5 shows an overlay of chromatograms of fresh honey (less than two weeks old, stored at 4 °C in a dark environment), a sample of honey from a lot that was rejected by quality control department due to off-taste, and a honey sample that has been stored in the warehouse where the air conditioning system failed and the sample was exposed to 40 °C for four weeks. Trace amounts of 2-furfural and 5-methyl-furfural were detected in the fresh samples, but all were below LOQ. The concentrations of furfurals in the rejected sample were substantially higher 35 ng/g (2-furfural) and 33 ng/g (5-methyl-furfural). In the honey sample where air conditioning for storage was breached, 2-furfural and 5-methyl-furfural were detected at the concentration of 6 ng/g and 12 ng/g respectively. These examples demonstrated the effectiveness of the method in identifying off-grade honey products.

4. Conclusions

A fast and practical analytical approach has been successfully developed and implemented for the determination of ultra-trace parts-per-billion level of 2-furfural and 5-methyl-2-furfural in Manuka honey. With an appropriate extraction solvent, the use of piston-cylinder based LLE substantially decreases extraction time; by a factor of 120 when compared to SPME and decreases solvent consumption by a factor of 25 when compared to conventional mechanical agitation liquid–liquid extraction technique. The DB-624UI column used in this investigation offers sufficiently high inertness that derivatisation was avoided.

The analytical system requires minimal maintenance and is suitable for remote site deployment. Under the analytical conditions established and with a practical LOQ of 5 ng/g, 100 samples can be analysed before septum/liner/o-ring replacements are needed. As a preventive measure, the pre-column can be replaced once every six months to maintain chromatographic fidelity.

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References

[1] P. Molan, R. Cooper, Honey and sugar as a dressing for wounds and ulcers, Trop. Doct. 30 (2000) 249–250.
[2] H. Tan, R. Rahman, S. Gan, A. Halim, S. Hassan, S. Sulaiman, K.K. Bs, The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey, BMC Complement. Altern. Med. 9 (2009) 34.
[3] P. Merckholl, Bacteria, biofilm and honey: a study of the effects of honey on 'planktonic' and biofilm-embedded chronic wound bacteria, Scand. J. Infect. Dis. 41 (2009) 341–347.
[4] S. Kilty, D. AlMutari, M. Duval, M. Groleau, J. De Nanassy, M. Gomes, Manuka honey: histological effect on respiratory mucosa, Am. J. Rhinol. Allergy 24 (2010) 63–66.
[5] R. Jenkins, N. Burton, R. Cooper, Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*, J. Antimicrob. Chemother. 66 (2011) 2536–2542.
[6] J. Chavez-Servin, A. Castellote, M. Lopez-Sabater, Analysis of potential and free furfural compounds in milk-based formulae by high-performance liquid chromatography: evolution during storage, J. Chromatogr. A 1076 (2005) 133–140.
[7] E. Gaspar, J. Lopes, Simple gas chromatographic method for furfural analysis, J. Chromatogr. A 1216 (2009) 2762–2767.
[8] A. Ramirez-Jimenze, B. Garcia-Villanova, E. Guerra-Hernadez, Hydroxymethylfurfural and methylfurfural content of selected bakery products, Food Res. Int. 33 (2000) 833–838.
[9] F.J. Morales, S. Jimenz-Perez, Study of hydroxymethylfurfural formation from acid degradation of the Amadori product in milk-resembling systems, J. Agric. Food Chem. 46 (1998) 3885–3890.
[10] E. Ferrer, A. Alegria, R. Farrer, P. Abellan, F. Romero, High-performance liquid chromatographic determination of furfural compounds in infant formulas: changes during heat treatment and storage, J. Chromatogr. A 947 (2002) 85–95.
[11] F. Lo Coco, C. Valentini, V. Novelli, L. Ceccon, High-performance liquid chromatographic determination of 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde in honey, J. Chromatogr. A 749 (1996) 95–102.
[12] O. Koreh, K. Ludanyi, K. Vekey, E. Javorszky, E. Molnar, K. Torkos, J. Borassay, Determination of furanic compounds in insulating oil by high performance liquid chromatography/mass spectrometry using atmospheric pressure chemical ionization, Rapid Commun. Mass Spectrom. 12 (1998) 1515–1519.
[13] T. Bosworth, S. Setford, R. Heywood, S. Saini, Pulsed amperometric detection of furan compounds in transformer oil, Anal. Chim. Acta 450 (2001) 253–261.
[14] I. Rezic, A. Horvat, S. Babic, M. Kastelan-Macan, Determination of pesticides in honey by ultrasonic solvent extraction and thin-layer chromatography, Ultrason. Sonochem. 12 (2005) 477–481.
[15] M. Cais, M. Shimoni, M. Moshe, Method and device for mass transfer operation in immunoassays and other applications, US Patent #4,545,231 (12.06.1984).
[16] I. Peleg, S. Vromen, An efficient novel device for solvent extraction, Chem. Ind. 15 (1983) 615–616.
[17] T. Parliment, A new technique for GLC sample preparation using a novel extraction device, Perfumer and Flavorist 11 (1986) 1–8.
[18] Y. Tsai, Y. Huang, J. Fang, P. Wu, Percutaneous absorption of capsaicin and its derivatives, Drug Dev. Ind. Pharm. 20 (1994) 719–730.
[19] T. Guerin, I. Kennedy, Distribution and dissipation of endosulfan and related cyclodienes in sterile aqueous systems: implications for studies on biodegradation, J. Agric. Food Chem. 40 (1992) 2315–2323.
[20] K. Gras, R. Gras, J. Luong, T. Hayward, in: CASSS (Ed.), A High Performance GC Capillary Column for Challenging Industrial Applications, Proceedings of the 37th International Symposium on Capillary Chromatography, Poster, Palm Springs, CASSS Publisher, Palm Springs, 2013.
[21] M. Klee, Optimizing capillary column backflush to improve cycle time and reduce column contamination, J. Sep. Sci. 32 (2009) 88–98.